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Lost in translation? Multi-metric macrobenthos indicators and bottom trawling.

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Abstract

The member states of the European Union use multi-metric macrobenthos indicators to monitor the ecological status of their marine waters in relation to the Water Framework and Marine Strategy Framework Directives. The indicators translate the general descriptors of ecological quality in the directives into a single value of ecological status by combining indices of species diversity, species sensitivity and density. Studies and inter-calibration exercises have shown that the indicators respond to chemical pollution and organic enrichment, but little is known about their response to bottom trawling. We use linear mixed effects models to analyze how bottom trawling intensity affects the indicators used in the Danish (Danish Quality Index, DKQI) and Swedish (Benthic Quality Index, BQI) environmental monitoring programs in the Kattegat, the sea area between Sweden and Denmark. Using year and station as random variables and trawling intensity, habitat type, salinity and depth as fixed variables we find a significant negative relationship between the BQI indicator and bottom trawling, while the DKQI is related significantly to salinity, but not to trawling intensity. Among the indicator components, the species diversity and sensitivity indices used in the DKQI are not significantly linked to trawling, and trawling only affects the BQI when species sensitivities are derived from rarefied samples. Because the number of species recorded per sample (species density) is limited by the number of individuals per sample (density), we expect species density and density to be positively correlated. This correlation was confirmed by a simulation model and by statistical analysis of the bottom samples in which log species density was highly significantly related to log density ($r=0.75$, $df=144$, $p<0.001$). Without accounting for the effect of density on species density, indicators based on species density will be affected by temporal and spatial variations in density linked e.g. to variable recruitment

33 success. When this variation is accounted for by random year and station effects we find log
34 trawling intensity to explain more of the variation in log density than in the indicators
35 currently used to monitor Good Ecological and Environmental Status in the Kattegat.
36 Disregarding random effects and the relationship between density and species density, the
37 impacts of bottom trawling are likely to be lost in the translation of ecological quality into
38 macrobenthos indicators.

39 Keywords: macrobenthos indicators, bottom trawling, density, species richness, Water
40 Framework Directive, Marine Strategy Framework Directive

41

42 1. Introduction

43 Quantification of the ecological status of marine soft-bottom macrobenthos has become
44 increasingly important in Europe after the implementation of the European Water
45 Framework Directive (WFD; 2000/60/EC) and the European Marine Strategy Framework
46 Directive (MSFD; 2008/56/EC). Both directives contain descriptors of ecological quality and
47 require the status of marine macrobenthos to be assessed and expressed relative to a
48 situation where anthropogenic impacts are either negligible or at a sustainable level (Van
49 Hoey *et al.* 2010, Borja *et al.* 2013). However, translating the qualitative descriptors in the
50 directives into quantitative measurable ecological and environmental properties is an ongoing
51 challenge (Van Hoey *et al.* 2010). So far the translation has relied heavily on the use of
52 ecological quality indicators which have been used to express the current ecological and
53 environmental status in relation to the desired (Rice *et al.* 2012, Birk *et al.* 2012). The main
54 purpose of these indicators is to link a specific anthropogenic pressure to a change in
55 ecological quality extracted from a multivariate response (Hiddink *et al.* 2006, Muntadas *et al.*
56 2016, Rijnsdorp *et al.* 2016). The link between pressure and response is important because
57 the likelihood that managers will act to reduce or remove ecologically adverse pressures
58 depends on the quality and strength of the scientific evidence that action will result in the
59 outcome intended. Without a scientifically well documented causal relation between a
60 particular pressure and ecological status, managers may be less likely to regulate ecologically
61 adverse pressures, in particular if these pressures are generated by human activities that are
62 economically, politically or socially important. Examining how well indicators link pressure to
63 state is therefore important.

64 The member states of the European Union have been granted considerable flexibility
65 regarding the implementation of the WFD in their national marine waters and, as a result,
66 many have selected their own indicator to quantify the status of their soft-bottom
67 macrobenthic invertebrate fauna (Quintino *et al.* 2006, Borja & Dauer 2008, Pinto *et al.* 2009,
68 Josefson *et al.* 2009, Birk *et al.* 2012, Borja *et al.* 2015). Most of these indicators address the
69 normative definitions and terms of the WFD and therefore include estimators of ‘the level of
70 diversity and abundance of invertebrate taxa’ and the proportion of ‘disturbance-sensitive
71 taxa’ (Vincent *et al.* 2002, Borja *et al.* 2004). In practice, this means that they combine a
72 diversity index with an expression of the number of individuals or species present in each

73 sample and a formula reflecting the observed relative occurrence or abundance of
 74 disturbance-sensitive macrobenthic taxa. To assess the relative occurrence of disturbance-
 75 sensitive taxa the majority of the member states use the AZTIs Marine Biotic Index (AMBI)
 76 (Borja *et al.* 2000), and a few use the sensitivity metric in the Benthic Quality Index (BQI)
 77 (Rosenberg *et al.* 2004), or other metrics. To reflect ‘the level of diversity’ Shannon’s diversity
 78 index (H') (Shannon & Weaver 1963) is often used, and to reflect ‘abundance of invertebrate
 79 taxa’, either the number of species recorded or a combination of species recorded and
 80 individual density is most often used (Borja *et al.* 2009). Hence, sensitivity as defined by AMBI
 81 or by the BQI, diversity as reflected by H' , and some function of the number of species
 82 recorded or density are the most common metrics incorporated in the indicators.

83 Most of the development, testing and inter-calibration of the national macrobenthos
 84 indicators have focused on their response to eutrophication, organic enrichment and chemical
 85 pollution (Borja *et al.* 2007, Borja *et al.* 2015), and comparatively little work has been spent
 86 on examining their response to bottom trawling and seabed abrasion. This is problematic
 87 because fisheries generated abrasion exerts a significant pressure on soft-bottom
 88 macrobenthic communities in many areas (Kaiser *et al.* 2006, Collie *et al.* 2016, Eigaard *et al.*
 89 2016, 2017). Furthermore, the response of the benthic fauna to mechanical abrasion may very
 90 well differ from its response to eutrophication, organic enrichment and chemical pollution.
 91 According to the widely accepted ‘Pearson and Rosenberg model’, organic enrichment will
 92 initially increase the growth, density and species richness of the macrobenthos (Pearson &
 93 Rosenberg 1978, Gray *et al.* 2002). A further increase in organic enrichment will increase the
 94 oxygen uptake of the seabed eventually resulting in hypoxia or anoxia and a decline in species
 95 richness due to a reduction in density or disappearance of sensitive species unable to thrive at
 96 low oxygen concentrations. In contrast, mobile bottom-contacting fishing gears are known to
 97 kill or damage organisms that are sensitive to mechanical abrasion (Kaiser *et al.* 2006, Clark *et al.*
 98 2016, Collie *et al.* 2016, Neumann *et al.* 2016). A single passage of a bottom trawl will
 99 typically kill 20–50% of the benthic invertebrates in the path of the gear (Collie *et al.* 2016),
 100 but the response is variable and depends on the type of habitat (e.g. substrate), the level of
 101 natural disturbance (e.g. hydrographic regime), the species composition of the benthic
 102 community, and the footprint of the gear in use (Kaiser *et al.* 2006, van Denderen *et al.* 2014,
 103 2015, Eigaard *et al.* 2016, 2017). Where the longer term response of soft-bottom marine

104 macrobenthos to organic enrichment is expected to be a uni- or bi-modal change in benthos
105 biomass, density and species richness, the response to an increase in bottom trawling seems
106 more likely to be a monotonic decline in the biomass and density of sensitive organisms that
107 are sampled by bottom corers and grabs (Queirós *et al.* 2006, Hinz *et al.* 2009).

108 There is, however, a fundamental, but frequently neglected problem that can compromise the
109 assessment of biodiversity with bottom corers and grabs. A single sample represents a fixed
110 sampling area and provides an estimate of species density (the number of species per
111 sampling area), and not species richness (the total number of species present in the habitat
112 sampled). Estimates of species density are often highly correlated with the number of
113 individuals recorded in the samples. This correlation is known to complicate analyses of
114 changes in species density (Gotelli & Collwell 2010, Chase & Knight 2013). For instance, if a
115 sample only contains ten individuals, no more than ten species can be identified, irrespective
116 of the total number of species that are actually present in the habitat sampled. Hence, when
117 density changes at a particular location due to e.g. natural fluctuations in recruitment success
118 or increased mortality caused by bottom trawling, the number of individuals contained in
119 each sample will change, and so will the number of species recorded. A change in the number
120 of species recorded can thus be produced both by a change in the number of species occurring
121 at the location and by a change in the density of individuals affecting how likely it is that the
122 species are represented in the samples. Most macrobenthic indicators use species density to
123 quantify ecological quality and may therefore respond to changes in individual density and
124 distribution as well as to the number of species present.

125 The purpose of this study is therefore twofold: To investigate the response of the current
126 macrobenthos indicators to bottom trawling; and to examine how the link between species
127 density and individual density may affect the indicators. To this end we analyze a dataset from
128 the Danish macrobenthos monitoring program in the Kattegat between Denmark and Sweden.
129 We focus on the response of the multi-metric DKI and BQI indicators used to monitor
130 macrobenthos quality by the two countries in relation to the Water Framework Directive.
131 Both indicators contain similar elements as the majority of macrobenthos indicators used by
132 other EU member states. Using mixed effects models and estimates of trawling intensity
133 around the benthos sampling stations we investigate how the indicators and their
134 components respond to trawling intensity using salinity, habitat type, and depth as co-

135 variates and station and year as random effects. Finally, we discuss how to evaluate the
136 ecological status of macrobenthic communities in relation to bottom trawling and other
137 anthropogenic pressures.

138

139 2. Material and Methods

140 2.1 Study area

141 The Kattegat is situated between Sweden and Denmark and has a total area of $\sim 22000 \text{ km}^2$
142 (Figure 1). Most of the western part is relatively shallow and sandy with depths between 10
143 and 20 m, but the northern and eastern parts comprise a complex postglacial seascape with
144 deep muddy canyons down to 150 m in between shallower mounts of mixed sediments and
145 reefs formed by leaking gases (Al'Hamdani *et al.* 2007). The Kattegat connects the saline
146 North Sea (salinity $>30 \text{ ppm}$) with the more brackish Baltic Sea ($<20 \text{ ppm}$) and exhibits a
147 strong vertical stratification as well as a horizontal salinity gradient where salinity below the
148 halocline declines from 34 ppm in the north to 28 ppm in the south. An intensive bottom trawl
149 fishery for Norway lobster (*Nephrops norvegicus*) impacts the deeper ($\geq 16 \text{ m}$) soft-bottom
150 macrobenthic communities (Pommer *et al.* 2016). In the more shallow sandy areas, a now
151 much reduced bottom trawl fishery for plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*)
152 takes place (Svedäng *et al.* 2010, Cardinale *et al.* 2010). The Kattegat has been subject to
153 eutrophication and suffered from hypoxic and anoxic events in the 1980's, but since then the
154 amount of nutrients from land has been reduced and the frequency of hypoxic events has
155 declined (Riemann *et al.* 2016).

156 2.2 Benthos samples

157 Benthos was sampled annually on 22 fixed stations using a Haps corer covering an area of
158 0.0143 m^2 (Kannevorff & Nicolaisen 1973). At each station five replicate Haps samples were
159 collected in April or May in the years 2005-2008, 2010, 2011 and 2013 (Figure 1). Each Haps
160 sample was carefully flushed through a 1 mm mesh sieve to extract the animals, which were
161 preserved in a 96 % ethanol solution (Josefson and Hansen 2014). In the laboratory, all
162 individuals were sorted and identified to the lowest possible taxon, preferably to the species
163 level, and the number of individuals of each species or taxon was counted. To reduce the

164 variance the five Haps samples from each station were combined prior to the calculation of
165 the DKI and BQI indices. At each station estimates of the average near bottom salinity, depth
166 and sediment type at EUNIS (European Nature Information System) habitat level 3 were
167 available.

168 2.3 Trawling intensity

169 The area swept by trawling was estimated within a circle with a radius of 2 km centered at
170 each benthos station. Recruitment of most benthic species in the area takes place from early
171 spring until late autumn and many of the organisms present in the samples in late April or
172 early May will be surviving recruits from the previous year. At each station trawling intensity
173 was therefore cumulated over the period from May in the preceding year to April in the year
174 where the bottom samples had been collected. The area swept was estimated by combining
175 data from the Danish Vessel Monitoring System (VMS) with logbook data and estimates of the
176 towing speed and dimensions of the trawl gears that had been used. Before 2012, the VMS
177 was only mandatory for vessels longer than 15 m, but although some smaller bottom trawlers
178 fish in the Kattegat, vessels ≥ 15 m constitute by far the largest part of the bottom trawlers
179 (Danish AgriFish Agency 2016). Vessel speed was used to separate actively fishing vessels
180 from steaming and idle vessels. To calculate the footprint for each logbook-registered fishing
181 trip, we used the relationships between gear dimensions and vessel size (e.g. trawl door
182 spread and vessel engine power (kW)) from Eigaard *et al.* (2016) for different gear types,
183 vessel groups and target species. Combined with vessel tracks based on the VMS positions and
184 the interpolation method of Hintzen *et al.* (2010) these data were used to calculate trawling
185 intensity, defined as the ratio of the annual area swept to the size of the circular area
186 surrounding each station. The average trawling intensities ranged from 0 times per year to 73
187 times per year at the stations in the central part of Kattegat (Figure 1).

188 2.4 Macrobenthos Quality Indicators

189 The current version of the Danish Quality Indicator (DKI) is described in Henriksen *et al.*
190 (2014). It combines the AMBI index of Borja (2000), where species or taxa are classified
191 according to their sensitivity to organic enrichment and pollution, the number of individuals
192 N , and Shannons diversity index H' , calculated using \log_2 . The AMBI and Shannon indices

193 were both standardized by means of empirical salinity regressions derived from another set
194 of reference samples (Table1).

195 The Benthic Quality Index (BQI) was calculated from the formula presented in Leonardsson *et*
196 *al.* (2009), who also presents sensitivity values for a range of species estimated from a large
197 collection of reference samples in the Kattegat and Skagerrak. The assumption behind the BQI
198 index is that sensitive species can be characterized by occurring in samples with a high
199 number of species, while tolerant species are found in samples with a low number of species
200 (Rosenberg *et al.* 2004). After using the formula of Hurlbert (1971) to calculate the expected
201 number of species to be found in rarefied reference samples of 50 individuals, the sensitivity
202 of species i , $Sens_{E,i}$, is estimated as the lower 5 % percentile of the expected number of
203 species found in all the reference samples in which species i is present. A high sensitivity
204 value thus signifies that a species would tend to occur in areas of high species density.

205 Because the sensitivity of a species in the BQI index is determined from its relative occurrence
206 in the reference samples, sensitivity will depend on the number and mixture of reference
207 samples available from disturbed and undisturbed environments. When Leonardsson *et al.*
208 (2015) updated the sensitivities used in Leonardsson *et al.* (2009) they included reference
209 samples dominated by high numbers of juveniles of one or two species. The high numbers of
210 juveniles in these samples were found to decrease the sensitivity estimates of the other
211 species represented in the samples. Leonardsson *et al.* (2015) therefore decided to abandon
212 rarefaction in the sensitivity calculation, and changed the base for calculating the sensitivities
213 from the rarefied number of species to the observed number of species. The new species
214 sensitivity, $Sens_{O,i}$, was defined as the 5th percentile of the observed number of species each
215 individual of species i encountered in the reference samples where i was present
216 (Leonardsson *et al.* 2015). To examine the effect of this approach we also estimated the BQI
217 indicator $BQI_{O,j}$ for each sample based on $Sens_{O,j}$, the weighted sum of the revised species
218 sensitivities, $Sens_{O,i}$, provided by Leonardsson *et al.* (2015).

219

220 2.5 Statistical modeling

221 All variables were initially examined by pairwise plots and Pearson correlations to reveal the
222 shape of potential relationships and the patterns of interaction. An analysis of covariance was
223 then used to assess the relative importance of the variables used to calculate the DKI and BQI
224 indicators and the Shannon index, while log linear mixed effects models were used to analyze
225 the relationships between the indicators and environmental variables. The log linear mixed
226 effects models used log trawling intensity, EUNIS habitat, log depth and log salinity as fixed
227 effects while station and year were assumed to be random effects considered to reflect
228 random differences in community attributes between stations as well as random inter-annual
229 changes in benthic recruitment success. The analyses of the mixed models were performed in
230 R (R Core Team 2015) using the lme4 and lmerTest packages (Bates *et al.* 2015). Residual
231 plots and Q-Q plots were inspected for deviations from homoscedasticity and normality. If
232 necessary, variables were $\log(x+1)$ rather than log transformed to include zero observations.
233 Parameter estimates were obtained using restricted maximum likelihood and significant
234 variables were identified using backwards elimination of model terms. Alternative model
235 versions were compared using maximum likelihood and Bonferroni adjusted likelihood ratio
236 tests. Only natural logarithms were used.

237 The initial correlation analysis revealed a linear and highly significant relationship between
238 log density and log species density ($r=0.75$, $df=144$, $P<0.001$, Figure 3) indicating that it was
239 necessary to standardize species density to account for differences in the number of
240 individuals recorded per sample across stations and years.

241 When only a small fraction of the individuals in a habitat or community is sampled, the
242 number of species recorded provides an underestimate of total species richness which is
243 biased against rare species. This problem was first described for marine benthos by Sanders
244 (1968) and is often solved by individual-based rarefaction where the number of species
245 observed is standardized to the expected number of species observed in a sample containing
246 the same number of individuals, n , as the smallest sample in the group of samples being
247 compared. Rarefying a sample from N to n individuals can mathematically be solved as a
248 combinatorial problem providing an analytical formula for estimating the expected number of
249 species in a random sample of n individuals drawn from a larger N individual sample
250 (Hurlbert 1971, Heck *et al.* 1975). This, however, assumes that the spatial distribution of the
251 individuals in the environment is random. If the spatial distribution is patchy, rarefaction of

252 large samples tends to overestimate the number of species in small samples (Gotelli & Colwell
253 2011). Previous investigations have found that the distribution of benthos in the Kattegat is
254 patchy (Josefson 2016). Furthermore, our samples contained between 15 and 547 individuals
255 necessitating us to rarefy all samples to 15 individuals. Instead of using rarefaction to
256 standardize the number of species prior to our statistical analysis we therefore decided to
257 include a species accumulation curve directly in the statistical model.

258 A species accumulation curve describes the curvilinear relationship between the number of
259 individuals sampled and the number of species identified. Following the approach of Azovsky
260 (2011) we used a power function to describe this relationship and linearized it by using log
261 species density and log density in the analysis. This allowed us to use the linear mixed effects
262 model to investigate whether trawling intensity significantly affected the relationship. Note
263 that a species accumulation curve generally is used to express the relationship between the
264 number of species identified and the cumulative number of samples or individuals examined
265 from a particular habitat or community (see Gotelli & Colwell 2001). Here we assume that a
266 single species accumulation curve can be used to model samples from different locations and
267 environmental conditions when environmental covariates and random effects of year and
268 stations are simultaneously accounted for.

269 To examine how removal of species and individuals due e.g. to trawling might affect the shape
270 of the accumulation curve, we also developed a simple stochastic benthic community model
271 where a lognormally distributed species density distribution was randomly generated for 100
272 species, using the same mean and standard deviation as found in the samples (mean=1.3,
273 stdev=1.3). We then sequentially removed the most abundant, the least abundant, or a
274 randomly selected species from the community and fitted species accumulation curves to the
275 results. We also investigated the effect of removing different proportions of the individuals
276 from all of the species.

277

278 3. Results

279 The pairwise plots and Pearson correlations reveal important and significant linkages
280 between the independent and dependent variables. The most significant interactions are

presented in Figures 2 to 4 and the full correlation matrix is shown in the supplementary material (Figure S1). $\log N_j$, $\log S_j$, DKI_j , $BQI_{E,j}$, and $Sens_{E,j}$ were all negatively related to log trawling intensity, while $Sens_{O,j}$ was significantly positively correlated to log trawling intensity, and the $Shannon_j$, $AMBI_j$, and $BQI_{O,j}$ indices did not change significantly with log trawling intensity (Figure 2). Log species density, $\log S_j$, and log density, $\log N_j$, were highly significantly positively correlated (Figure 3). Furthermore, trawling intensity and BQI_O were positively related to salinity, while both DKI and BQI_E declined with salinity (Figure 4).

The analysis of covariance showed that 54 % of the observed across sample variation in the DKI indicator was attributed to variation in the Shannon index, and 37 % was attributed to salinity (Table 2). The Shannon index was dominated by changes in S which explained 69 % of the variation of the index. The variation of the BQI_E was significantly related to changes in both $\log S_j$ and in the Sensitivity index, $Sens_E$, which explained 68 % and 27 % of the variation in the indicator, respectively. The same two indices affected the BQI_O where they explained 56 % and 40 % of the variation, respectively.

The linear mixed effects model confirmed that $\log N$ was highly significantly negatively related to log trawling intensity (Table 3). This effect was not just caused by a few stations. Removing the random station effect from the model and estimating a separate slope for each station revealed that $\log N$ declined with log trawling intensity on 18 of the 22 stations, and that the decline was statistically significant for 11 stations. $\log S$ was found to be linearly and highly significantly positively related to $\log N$, but not to log trawling intensity, nor to any of the other environmental variables. There was furthermore no significant interaction between the slope of this relationship and log trawling intensity. The linear mixed-effects model showed that the DKI indicator responded significantly to salinity, while the $AMBI$ and $Shannon$ indices neither responded significantly to log trawling intensity nor to any of the other environmental variables. The BQI_E and its associated sensitivity index both responded significantly to log trawling intensity, but not to log salinity or log depth (Table 3). This relationship disappeared when species sensitivities were based on the observed number of species. BQI_O did not respond significantly to any of the explanatory variables, while $Sens_O$ was highly significantly related to salinity. Table 4 provides a full account of the model

310 reduction including AIC-values (Akaikes Information Criteria; AIC) and significance of model
311 comparisons.

312 Using the stochastic benthic simulation model revealed that linear relationships between log
313 density and log species density could indeed be generated by removing either species or
314 individuals from a simulated assemblage. The linear relationships had slopes between 0.7 and
315 1.5 depending on whether species were removed at random or according to ranked
316 abundance (Figure 5). Removing a fixed proportion of the individuals from each species
317 generated a slope in the species abundance regression of 0.50, not significantly different from
318 the slope of 0.53 estimated from the data (Table 3).

319

320 4. Discussion

321 4.1 Indicator performance

322 The DKI indicator was found to be significantly negatively related to salinity, but not to
323 trawling. This response was puzzling, because neither the AMBI nor the Shannon index
324 responded significantly to any of the fixed variables included in the mixed effects model. The
325 significant salinity response of the DKI may, however, have been introduced by the salinity
326 standardization which was done without considering the potential effects of differences in
327 trawling intensity, eutrophication, and frequency of hypoxia events that could have influenced
328 density and species density at each of the reference sampling stations. Using salinity as the
329 sole explanatory variable in the standardization may produce a salinity corrected indicator
330 where salinity unintendedly provides the best explanation for the changes observed. In the
331 Kattegat, most of the *Nephrops* trawl fishery takes place below the halocline in the northern
332 deeper parts, where salinity is higher than in the shallower southern part, and salinity and
333 trawling intensity is therefore positively correlated ($r=0.52$, $df=146$, $P<2.72e-11$, Figure 4).
334 The standardization may thus inadvertently have removed the effect of bottom trawling and
335 explained it as an effect of salinity. Adding a log trawling intensity term to the reduced DKI
336 model where salinity was the only fixed term did not improve the goodness of fit (ANOVA,
337 $P=0.23$, $df=1$) although salinity and log trawling intensity are significantly and positively
338 related.

339 The only macrobenthos indicator that responded significantly to trawling intensity in the
340 linear mixed effects model was the BQI_E . The response was negative and highly significant
341 and was caused by a combination of declines in the average species sensitivity and in the
342 number of species recorded per station (Figure 2). The closely related BQI_O indicator did not
343 respond. The main difference between the two indicators is the way that species sensitivities
344 are calculated. The BQI_E uses species sensitivities based on rarefied species density estimates,
345 while the BQI_O uses the observed number of species without rarefaction. Whether or not to
346 rarefy the species density estimates in the sensitivity calculation has previously been subject
347 to some debate. Fleischer *et al.* (2007) found the BQI, as defined by Rosenberg *et al.* (2004), to
348 be sensitive to sampling effort and therefore recommended to rarefy all species density
349 estimates used in the formula, a practice subsequently followed e.g. by Fleischer &
350 Zettler (2009), Grémare *et al.* (2009) and Chuševé *et al.* (2016). Leonardsson *et al.* (2009),
351 however, retained the practice of only rarefying the species density estimates in the reference
352 samples used for estimating species sensitivities, but not the number of species recorded at
353 each station (BQI_E), while Leonardsson *et al.* (2015) decided not to rarefy any of the species
354 density estimates (BQI_O), because this led to very low sensitivity estimates for species
355 occurring in reference samples dominated by high numbers of juveniles of one or few species.
356 There may be reasons for using the observed number of species to calculate sensitivities
357 during the period when larvae settle and juveniles are abundant, but our results (see Figure
358 2) show that this can lead to a significant positive relationship between trawling intensity and
359 sensitivity, and therefore decrease the ability of the BQI indicator to monitor the impacts of
360 fisheries induced mortality. Using the revised unrarefied species sensitivity values from
361 Leonardsson *et al.* (2015), the abundance weighted overall sensitivity and indicator values
362 were no longer significantly related to trawling. We cannot distinguish whether this was due
363 to the inclusion of samples from the settling period, where the effect of local pressures at the
364 seabed such as bottom trawling may not yet have affected species densities, or whether it was
365 caused by using unrarefied reference samples.

366 The sensitivity, species diversity, and density components of the multi-metric indicators we
367 have analyzed are contained in most of the national quality indicators of marine
368 macrobenthos that are used to define and monitor the ecological status of coastal and marine
369 waters throughout Europe. However, the species diversity and in some cases also the

sensitivity indices depend on comparable estimates of species density across stations and years. Species density influenced the DKI indicator substantially through the Shannon index, and explained more than half the variation in the BQI_E indicator and the Shannon indices. Only the BQI_O indicator was more sensitive to changes in the weighted species sensitivities at each sampling station than to log species density.

We furthermore found log species density to be highly significantly related to log density. If density varies between years due to natural differences in larval recruitment, the indicators are likely to provide a variable background for estimating of how species diversity may respond to anthropogenic pressures acting on the seafloor, such as bottom trawling. Finally, the linear mixed effects model explained 78 % of the variation in the density data, and 72 % of the variation of the BQI_E (Table 4). Based on these results, we thus find the density of benthic invertebrates to be a better indicator of bottom trawling than any of the present indicators used to monitoring the ecological quality of soft-bottom macrobenthos in the Kattegat.

4.2 Methodological implications

It is often forgotten that quantitative sampling devices such as bottom grabs and corers only provide a count of the number of species per surface area sampled and not an un-biased estimate of the total number of species present in the habitat sampled (Gotelli & Colwell 2001). The difficulty arises because the number of individuals caught per sample limits the number of species that can be recorded per sample, generating a causal link between species density and individual density. When small bottom corers, such as the Haps, are used a typical sample may contain between 10 and 100 individuals, while more than 1000 benthic macroinvertebrate species have been recorded in the Kattegat and western Baltic (HELCOM 2012). Clearly only a fraction of these species will be recorded in a single sample. Exactly how many depends on the size of the local species pool, the spatial distribution of the individuals and/or species, and the number of individuals caught.

By simulating the relationship between species density and individual density in bottom samples we confirmed that the exponent of the species accumulation curve was sensitive to whether species were orderly or randomly removed. The slope in double-logarithmic plots of this relationship was steepest when the least abundant species were sequentially removed and shallowest when all species abundances were gradually reduced in the same proportion.

400 Interestingly, the slope generated by the analysis of the empirical data was not significantly
401 different from the slope generated by simulating a proportional reduction in abundance for all
402 species (Figure 5d).

403 Log species density and log density were both highly significantly correlated to each other and
404 to trawling intensity, but trawling did not seem to affect log species density above the effect
405 generated by its reduction of log density. When log density was included in the model of log
406 species density, the impact of trawling intensity on log species density was no longer
407 significant. There was also no significant effect of trawling intensity on the slope of the
408 relationship between log density and log species density. This suggests that log species
409 density is negatively affected by trawling simply because trawling reduces the density of
410 individuals. Had trawling affected the most abundant species more than the less abundant the
411 slope of the relationship between log density and log species density would probably have
412 steepened in response to trawling as shown by the simulations. The slope at the base of the
413 rarefaction curve has been shown to be equivalent to Hurlbert's probability of interspecific
414 encounter, which is a common sample size independent measure of evenness (Olszewski
415 2004, Chase & Knight 2013). Hence, because a rarefaction curve would correspond to the
416 lower part of the species accumulation curve a constant logged species accumulation slope
417 suggests that evenness is unaffected by fishing.

418 Although the slope of the log species accumulation curve thus appears to be resilient to
419 trawling, several decades of trawling could nevertheless have led to a gradual change in
420 species composition that would be important to monitor, but difficult to identify with the
421 present indicators. For instance, if changes in trophic interaction and interspecific
422 competition resulted in species replacements, but the overall relationship between density
423 and species density remained the same, indicators neglecting species identity might not
424 respond. However, previous investigations in the Kattegat have not suggested that species
425 replacements are likely to have happened. These investigations found inter-annual changes in
426 benthos abundance and recruitment to affect all species and all investigated locations
427 similarly, and suggested that a common factor could be operating, perhaps linked to the
428 deposition of organic material on the seabed (Josefson 1987, Josefson *et al.* 1993) or to
429 general climatic oscillations (Tunberg & Nelson 1998). Furthermore, Pommer *et al.* (2016)
430 found no relationship between bottom trawling intensity and changes in macrobenthos

community composition in the Kattegat. Zettler *et al.* (2017) investigated a 30 year time-series of benthos data from the western Baltic and concluded that benthic communities were influenced by a multitude of environmental variables and did not appear to be tightly controlled by any single environmental driver even within a restricted spatial area. We conclude that this calls for including environmental drivers as well as random year and station effects in the analyses in order to make anthropogenic impacts identifiable on a background of substantial natural variation.

4.3 Perspectives

A new generation of indicators is now being developed for monitoring macrobenthos status in relation to bottom trawling and MSFD requirements. Some of these indicators are based on changes in species or trait compositions (e.g. longevity) (Hiddink *et al.* 2006, Eigaard *et al.* 2017), and may suffer from the same sampling problems as the classical species density and diversity based indicators used to assess Good Ecological Status in relation to the WFD. We hope to have demonstrated that mixed effects models provide a possibility for dealing with some of these problems and allow a more precise translation of the qualitative descriptors of the directives into quantitative measurable goals. Using linear mixed-effects models of density solves the problem of standardization across different sources of variation by allowing incorporation of random effects of e.g. space (station) and time (month, year), generated by station specific differences in environmental conditions and by inter-annual differences in recruitment success, as well as fixed effects generated by quantified variables such as salinity, depth, bottom habitat and trawling intensity. Incorporation of environmental covariates and random effects allows changes in density to be mechanistically linked to differences in anthropogenic and natural pressures. Direct effects of fisheries generated mortality on macrobenthos communities can potentially be separated from indirect effects by examining how e.g. growth or reproduction is affected by trawling intensity, providing a possibility for defining limit reference points of relative densities below which offspring production can no longer secure replacement.

Finally, relative density could prove useful as an indicator of bottom trawling in parallel with other indicators. The AMBI has been shown to respond consistently to organic enrichment and pollution (Borja *et al.* 2015), but has been found to be less responsive to physical

462 disturbance (Muxika *et al.* 2005). We found no significant correlation between AMBI and
463 either density ($r=0.062$, $p=0.46$, $df=144$), log density ($r=0.10$, $p=0.22$, $df=144$) or log trawling
464 intensity ($r=-0.002$, $p=0.98$, $df=144$) in the Kattegat data showing that although AMBI might
465 be used as an indicator of chemical pollution, eutrophication and organic enrichment it is
466 unaffected by trawling intensity. Using several uncorrelated indicators, each responding to a
467 specific pressure, might provide the most unequivocal translation of the impacts of
468 anthropogenic pressures to ecosystem status and could help managers prioritize the
469 measures needed to achieve Good Ecological and Environmental Status in relation to the WFD
470 and MSFD targets for soft-bottom macrobenthos communities.

471

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670 of transitional waters: Is interpretation more than speculation?. *PloS one*, 12(4),
671 e0175746.

672 Table 1. Formulas used to calculate the DKI and BQI indicators

<p>Danish Quality Indicator (DKI) Henriksen(2014)</p>	$DKI_j = \frac{\left[\left(1 - \left(\frac{AMBI_j - AMBI_{j,min}}{7} \right) \right) + \frac{H'_j}{H'_{j,max}} \right] \left[1 - \frac{1}{N_{j,total}} \right]}{2}$ <p>Where:</p> $H'_{j,max} = 2.117 + 0.086 * salinity_j$ $AMBI_{j,min} = 3.083 - 0.111 * salinity_j$
<p>Benthic Quality Index (BQI_E) based on sensitivity estimated from the rarefied number of species Leonardsson <i>et al.</i> (2009)</p>	$BQI_{E,j} = \left[\sum_{i=1}^{S_{j,classified}} \left(\frac{N_{j,i}}{N_{j,classified}} * Sens_{E,i} \right) \right] \log_{10}(S_{j,total} + 1) * \left(\frac{N_{j,total}}{N_{j,total} + 5} \right)$
<p>Benthic Quality Index (BQI_O) based on sensitivity estimated from the observed number of species Leonardsson <i>et al.</i> (2015)</p>	$BQI_{O,j} = \left[\sum_{i=1}^{S_{j,classified}} \left(\frac{N_{j,i}}{N_{j,classified}} * Sens_{O,i} \right) \right] \log_{10}(S_{j,total} + 1) * \left(\frac{N_{j,total}}{N_{j,total} + 5} \right)$
<p>Where:</p> <p>$N_{j,i}$ number of individuals that belongs to species i in sample j.</p> <p>$N_{j,total}$ total number of individuals in sample j.</p> <p>$N_{j,classified}$ total number of individuals of species with known sensitivity value in sample j.</p> <p>$S_{j,total}$ total number of species observed in sample j.</p> <p>$S_{j,classified}$ number of species with known sensitivity present in sample j.</p> <p>$Sens_{E,i}$ sensitivity of species i calculated from the expected number of species in reference samples rarefied to 50 individuals.</p> <p>$Sens_{O,i}$ sensitivity value of species i calculated from the observed number of species in reference samples.</p> <p>H'_j Shannon diversity index of sample j calculated using \log_2.</p> <p>$H'_{j,max}$ predicted maximum Shannon diversity in sample j given local salinity.</p> <p>$AMBI_j$ value of AZTIs Marine Biotic Index ($AMBI$) (Borja et al. 2000) in sample j.</p> <p>$AMBI_{j,min}$ predicted minimum value of $AMBI$ index in sample j given local salinity.</p> <p>$salinity_j$ near the bottom salinity measured at the sampling station.</p>	

674 Table 2. Analysis of covariance of the DKI, BQI and Shannon indicators.

Indicator	Variable	Degrees of freedom	Sum of Squares	F-value	P(>F)	% of Total Sum of Squares
<i>DKI</i>	<i>AMBI</i>	1	0.111	2584.0	<2e-16	8
	<i>H'</i>	1	0.728	16903.5	<2e-16	54
	<i>1/N</i>	1	0.006	134.9	<2e-16	0
	<i>salinity</i>	1	0.493	11474.9	<2e-16	37
	Residuals	141	0.006			0
<i>BQI_E</i>	<i>Sens_E</i>	1	112.3	966.289	< 2e-16	27
	<i>logS</i>	1	287.2	2470.158	< 2e-16	68
	<i>N</i>	1	6.3	54.304	1.29E-11	1
	Residuals	142	16.5			4
<i>BQI_O</i>	<i>Sens_O</i>	1	1045.9	2185.715	< 2e-16	56
	<i>logS</i>	1	726.2	1592.767	< 2e-16	40
	<i>N</i>	1	6.9	14.454	2.13E-04	0
	Residuals	142	68.0			4
<i>H'</i>	<i>N</i>	1	13.3	116.31	<2e-16	14
	<i>S</i>	1	64.5	565.93	<2e-16	69
	Residuals	143	16.3			17

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676

677

678 Table 3. Result from fitting a linear mixed effects model with Year and Station as random
679 variables and habitat, log(depth), log(salinity) and log(trawling intensity+1) as fixed
680 independent variables to various response variables. Only the significant parameter estimates
681 are included in the final models and table. Log stands for natural logarithm, standard error is
682 shown in brackets, grey area signifies not investigated. Significance: *:P<0.05; **:P<0.01;
683 ***P<0.001

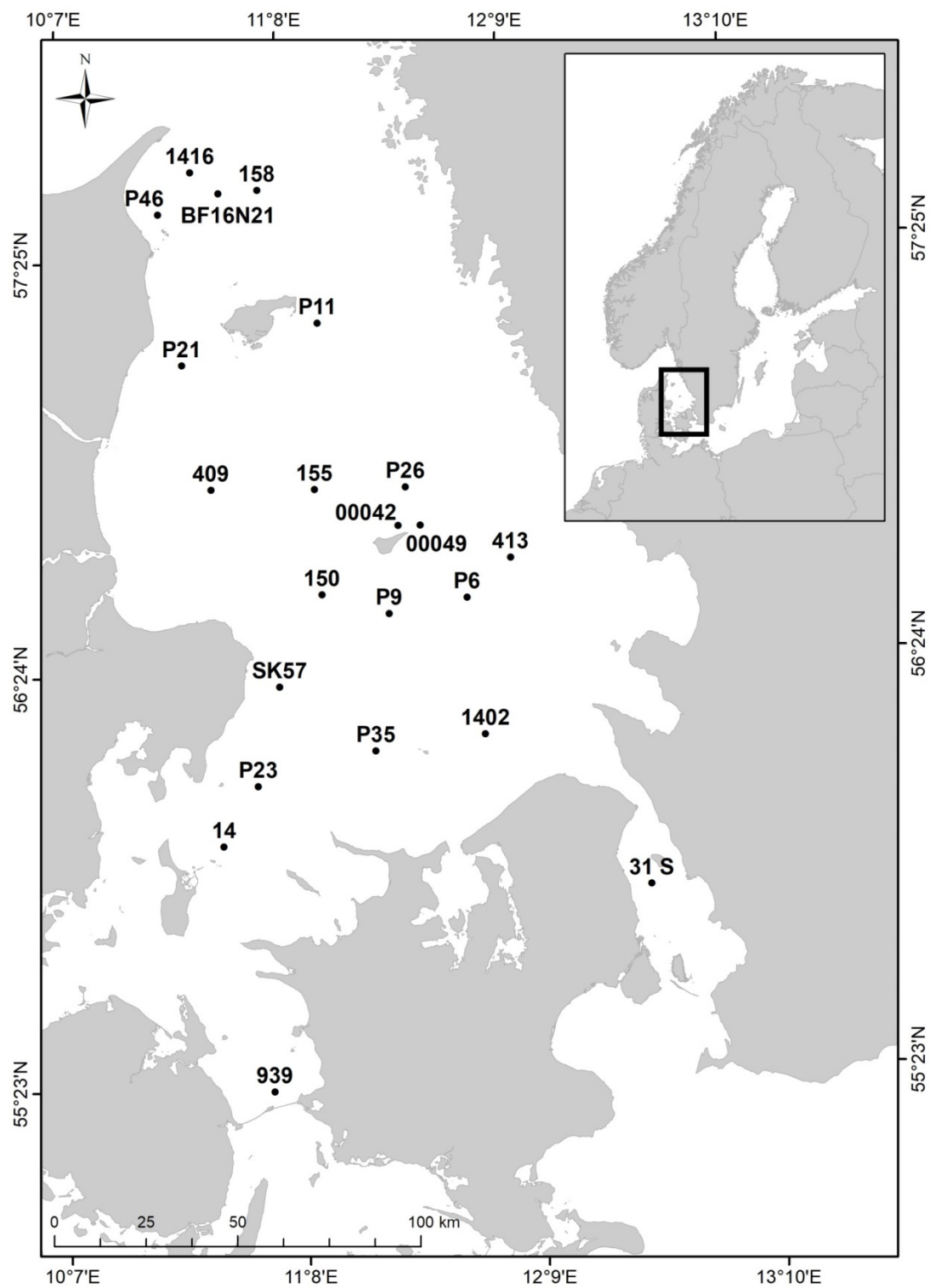
Response variable	Intercept	$\log N$	$\log(\text{salinity})$	$\log(\text{trawling} + 1)$
DKI	2.36(0.37)***		-0.48(0.11)**	
$AMBI$	1.71(0.06)***			
H'	3.44(0.15)***			
BQI_E	11.81(0.59)***			-1.14(0.32)***
$Sens_E$	8.71(0.15)***			-0.35(0.11)**
BQI_O	16.81(0.98)***			
$Sens_O$	-17.61(6.77)*		9.03(1.98)***	
$\log S$	0.67(0.21)**	0.53(0.04)***		
$\log N$	4.86(0.17)***			-0.29(0.09)**

684

685 Table 4. Backwards model reduction by removal of insignificant terms and likelihood ratio
686 tests. Selected models are shown in bold types. The R^2 is between predicted and observed
687 values. AIC is Akaike's Information Criteria and P is the probability from a likelihood ratio test
688 that the model explains the data significantly better than the previous model with the
689 additional term. Significance is Bonferroni corrected to account for the number of model
690 comparisons. *:P<0.05; **:P<0.01; ***P<0.001. Log stands for natural logarithm.

Model	R^2	AIC	P
$\log N = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.78	214.8	
$\log N = \log \text{trawl} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.78	211.8	0.383
$\log N = \log \text{trawl} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.78	210.3	0.469
$\log N = \log \text{trawl} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.78	209.8	0.230
$\log N = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.77	217.5	1.8e-3**
$\log S = \log N + \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.84	32.4	
$\log S = \log N + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.84	30.6	0.652
$\log S = \log N + \text{habitat} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.84	31.6	0.086
$\log S = \log N + \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.84	34.9	0.022
$\log S = \log N + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.84	32.9	0.258
$\log S = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.73	132.7	2.2e-16***
$DKI = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.83	-386.6	
$DKI = \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.83	-388.6	0.894
$DKI = \text{habitat} + \log \text{salinity} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.83	-387.0	0.058
$DKI = \log \text{salinity} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.83	-385.3	0.053
$DKI = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.83	-372.5	1.2e-4***
$AMBI = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.33	166.3	
$AMBI = \log \text{trawl} + \log \text{salinity} + \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.31	165.9	0.203
$AMBI = \log \text{trawl} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.30	165.8	0.114
$AMBI = \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.31	164.5	0.395
$AMBI = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.31	164.8	0.130
$\text{Shannon} = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.66	268.8	
$\text{Shannon} = \log \text{trawl} + \text{habitat} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.66	266.8	0.834
$\text{Shannon} = \log \text{trawl} + \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.66	267.5	0.104
$\text{Shannon} = \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.66	267.1	0.207
$\text{Shannon} = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.66	269.3	0.042
$BQI_E = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.73	580.7	
$BQI_E = \log \text{trawl} + \text{habitat} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.73	578.9	0.648
$BQI_E = \log \text{trawl} + \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.73	579.2	0.127
$BQI_E = \log \text{trawl} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.72	580.0	0.082
$BQI_E = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.72	590.0	5.2e-4**
$\text{Sens}_E = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.52	282.9	
$\text{Sens}_E = \log \text{trawl} + \text{habitat} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.52	284.0	0.078
$\text{Sens}_E = \log \text{trawl} + \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.52	286.1	0.043
$\text{Sens}_E = \log \text{trawl} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.52	284.8	0.194
$\text{Sens}_E = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.50	293.3	0.001**
$BQI_O = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.71	774.4	
$BQI_O = \log \text{trawl} + \text{habitat} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.71	772.1	0.107
$BQI_O = \log \text{depth} + \text{habitat} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.71	769.9	0.096
$BQI_O = \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.71	763.5	0.035
$BQI_O = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.71	760.1	0.219
$\text{Sens}_O = \log \text{trawl} + \text{habitat} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.76	529.4	
$\text{Sens}_O = \log \text{trawl} + \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.76	524.3	0.830
$\text{Sens}_O = \log \text{salinity} + \log \text{depth} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.76	523.0	0.405
$\text{Sens}_O = \log \text{salinity} + \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.76	527.0	0.014
$\text{Sens}_O = \varepsilon_{\text{station}} + \varepsilon_{\text{year}} + \varepsilon_0$	0.76	540.4	8.6E-5***

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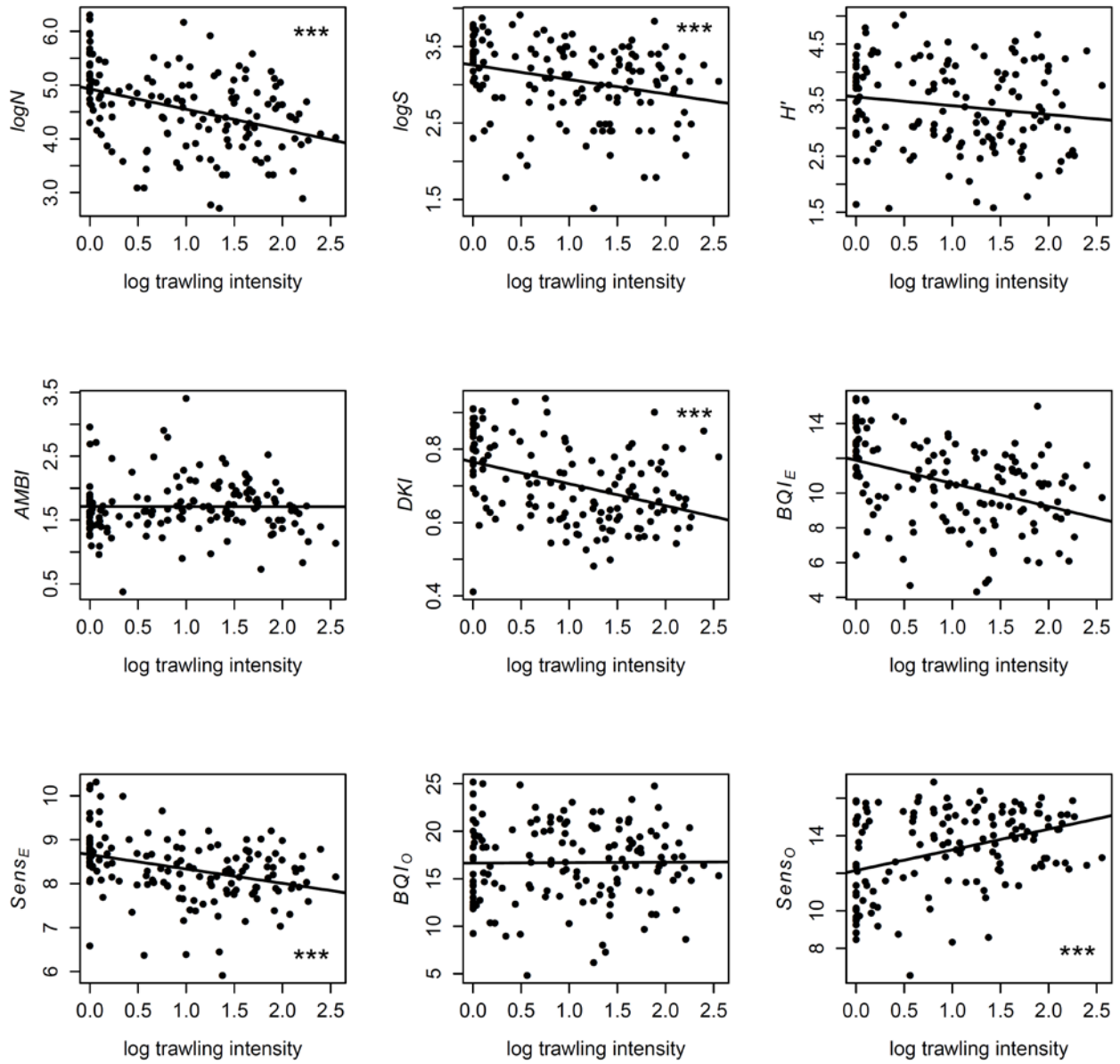


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693 Figure 1. Map of sampling stations.

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695



696

697

698 Figure 2. Linear regressions of $\log N$, $\log S$, H' , $AMBI$, DKI , BQI and Sensitivity versus log
 699 trawling intensity. Asterisks show level of significance: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

700

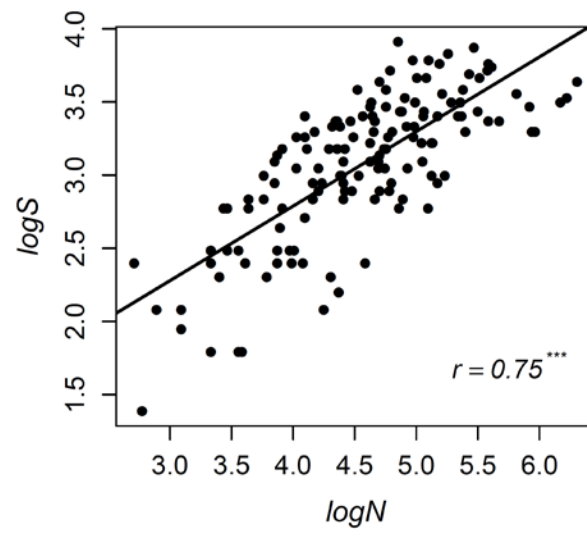
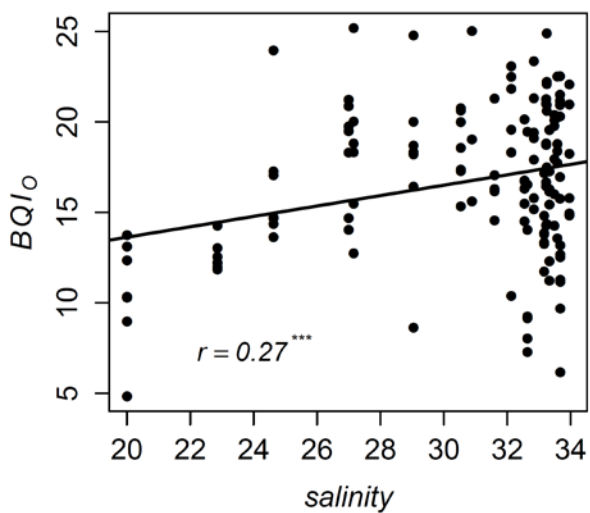
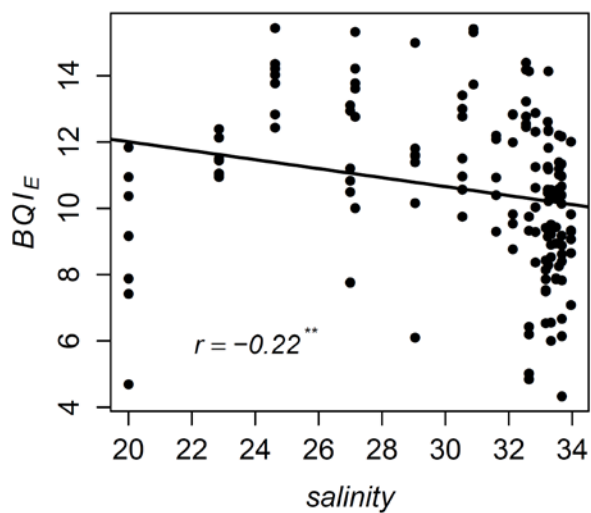
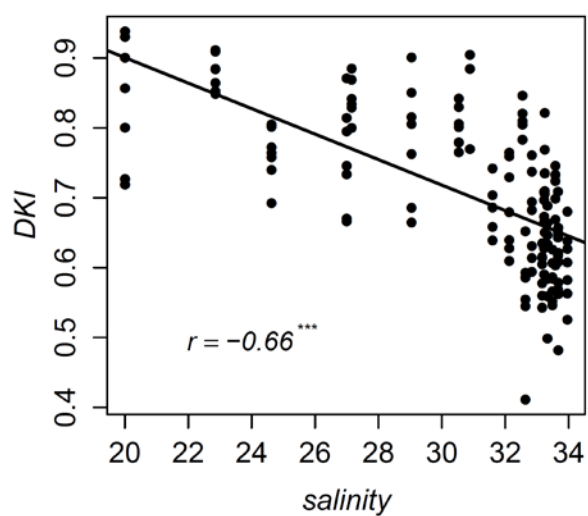
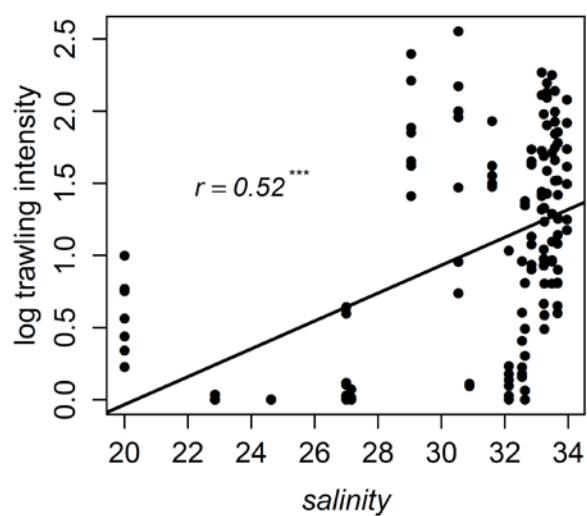
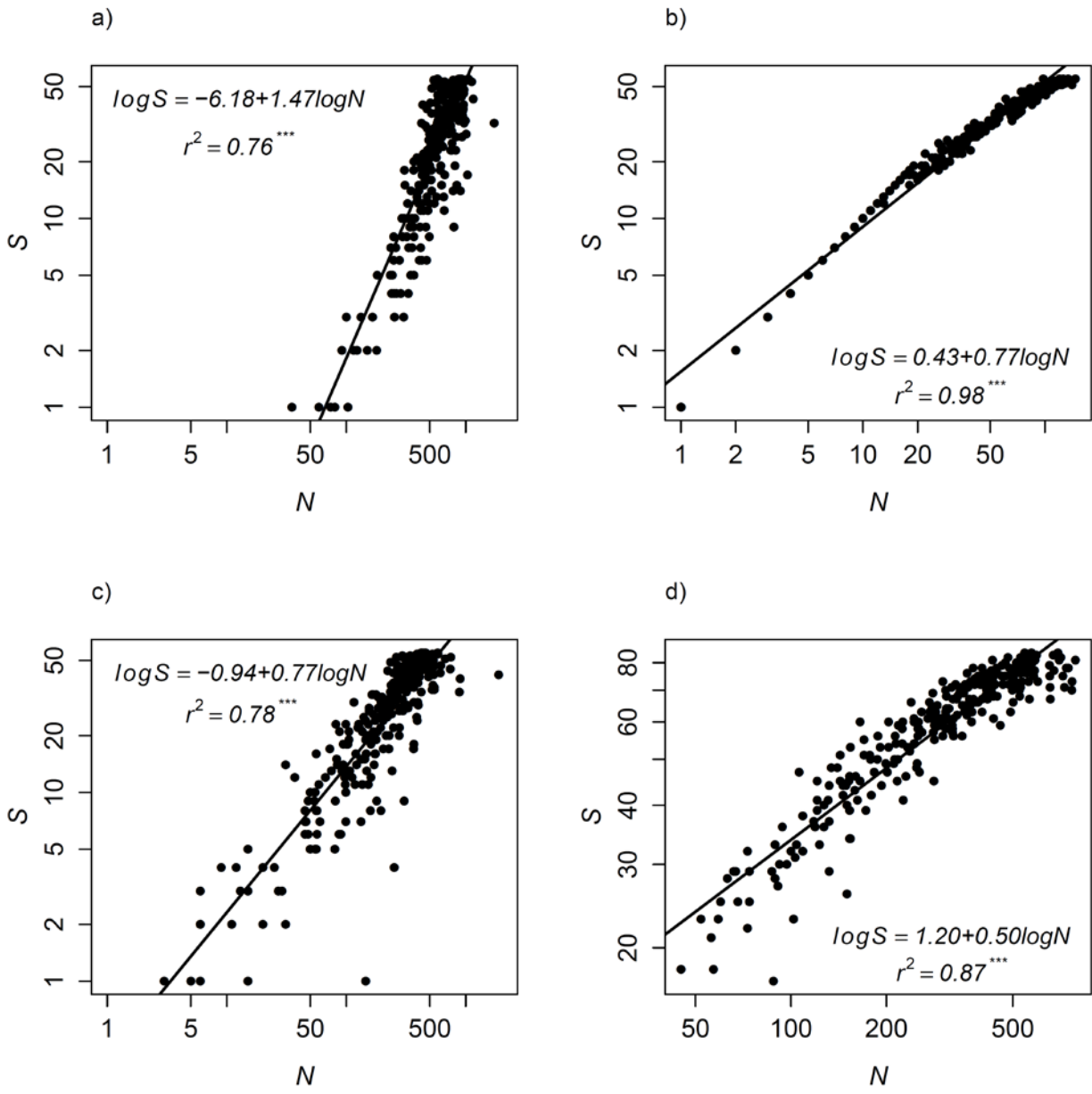


Figure 3. Linear relationship between log density and log species density.



704
705 Figure 4. Log trawling intensity, DKI, BQI_E and BQI_O versus salinity.



708 Figure 5. Simulated relationship between number of individuals and number of species.
709 Abundance of 100 species drawn at random from a lognormal distribution with a mean and
710 standard deviation of 1.3. Graphs show species abundance and number of species subject to a)
711 sequential removal of the least abundant species, b) sequential removal of the most abundant
712 species, c) random removal of species, and d) overall percentage reduction in abundance.

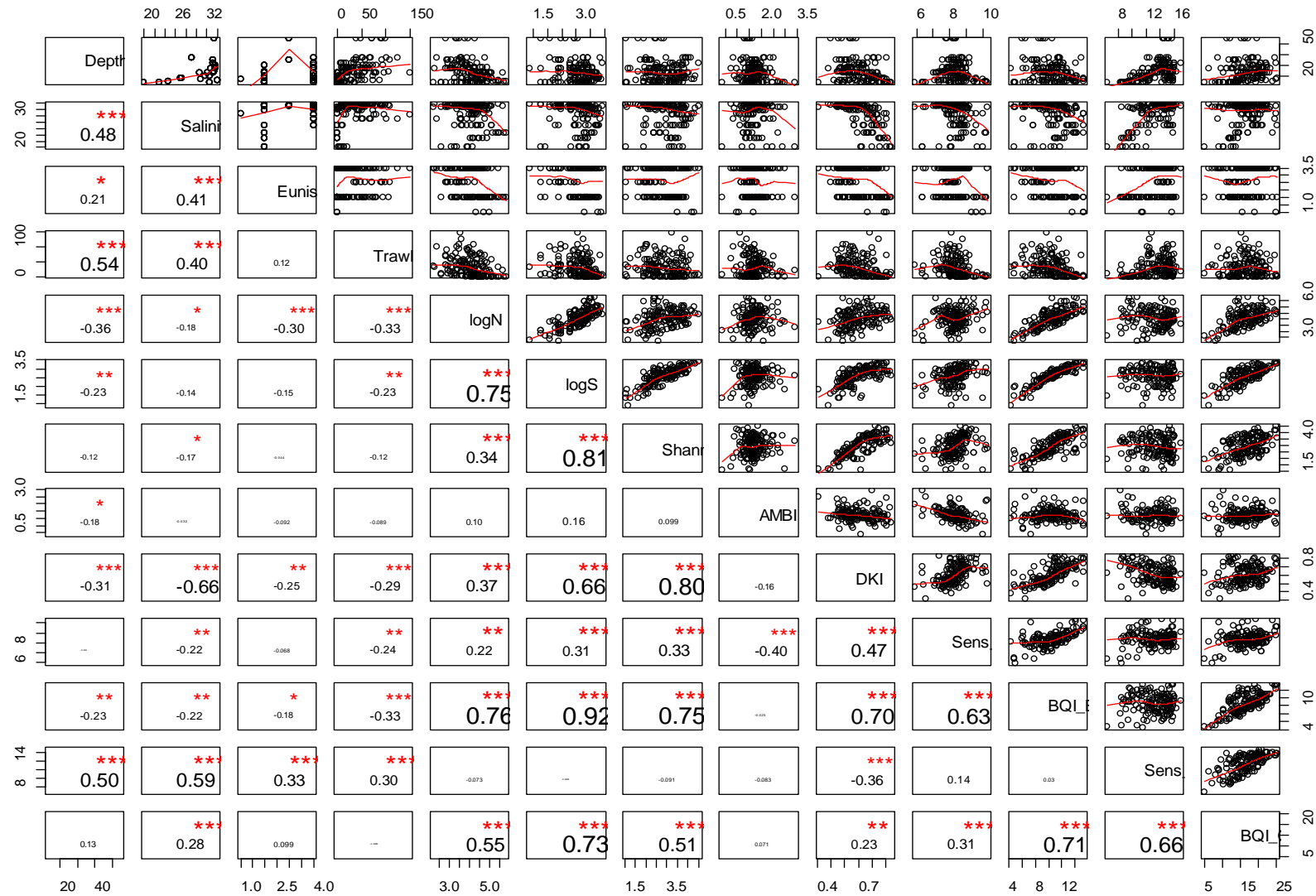


Figure S1. Pairs plot of dependent and independent variables with associated Pearson correlation coefficients. *:P<0.05; **:P<0.001; ***P<0.0001